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CIRCULAR DIFFERENTIAL SCATTERING CAN BE AN IMPORTANT PART OF THE CIRCULAR DICHROISM OF MACROMOLECULES (OPTICAL ACTIVITY/CIRCULARLY POLARIZED LIGHT/ NUCLEIC ACIDS)

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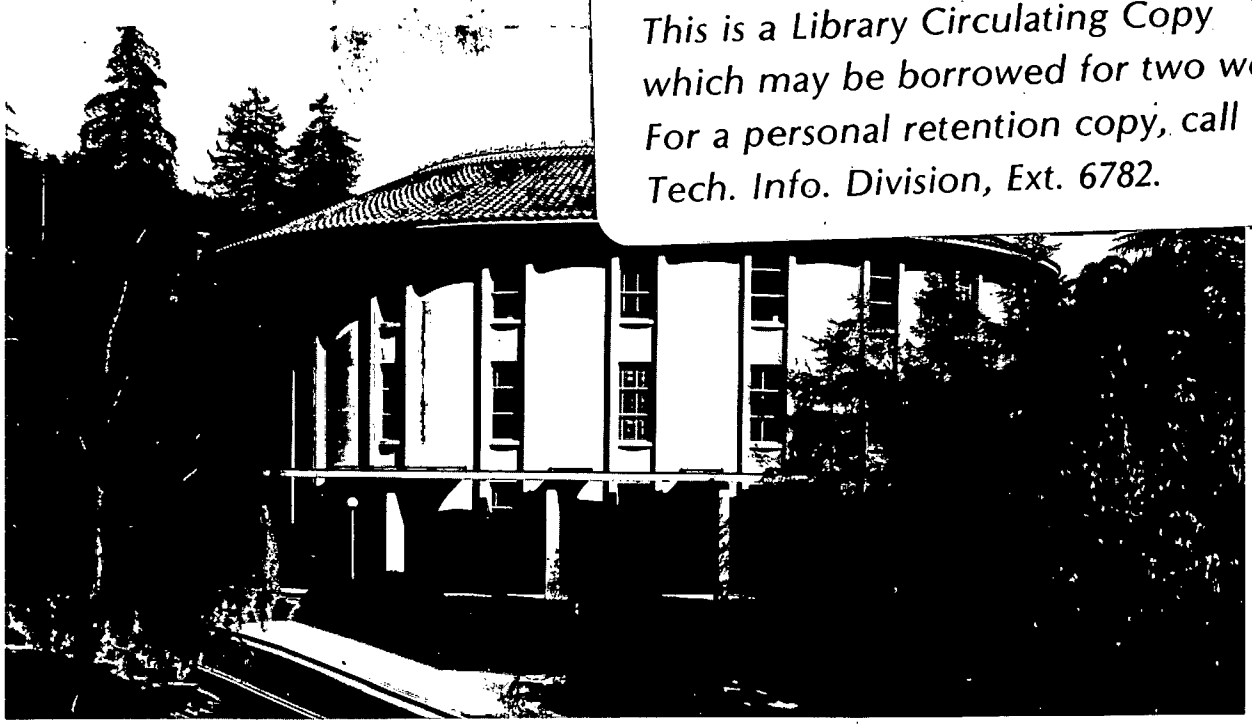

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DISCLAIMER

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Circular differential scattering can be an important part of the circular dichroism of macromolecules

(optical activity/circularly polarized light/nucleic acids)

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ABSTRACT Differential scattering of incident left and right circularly polarized light can be an important contribution to the circular dichroism of macromolecules. In principle both differential absorption and differential scattering of circularly polarized light contribute to circular dichroism, but differential scattering is increasingly important for particles whose dimensions are greater than one twentieth the wavelength of light. The scattering contribution is probably not important for unaggregated proteins and nucleic acids in solution. It can be very important for viruses, membranes and other protein-nucleic acid complexes. Outside the absorption bands of the scattering, chiral particle, only differential scattering contributes to the circular dichroism. The sign and magnitude of the differential scattering is quantitatively related to the relative orientations and the distances between the scattering units of the particle. The interpretation of the circular differential scattering depends on a simple, classical method. Thus, in understanding a measured circular dichroism, it will often be easier to relate the differential scattering to the structure of a particle (such as a virus) than it is to relate the differential absorption to the structure.

Circular dichroism (CD) and optical rotatory dispersion (ORD) studies have been very helpful in providing useful knowledge about the structure of biological macromolecules. These methods were originally limited to homogeneous solutions of macromolecules. More recently, they have also been applied to increasingly complex systems such as viruses (1,2), erythrocytes (3,4), nucleohistones (5,6), DNA-polylysine complexes (7,8), DNA aggregates (9), chloroplasts (10), etc. (11). A remarkable common feature of many of these systems was the presence of anomalies in their CD and ORD spectra. The CD spectra presented: (a) Apparent differential absorption of circularly polarized light outside of the absorption bands. The CD signal at long wavelengths was slowly varying, but non-zero; this long wavelength "tail" could be positive or negative. (b) Signals sensitive to the distance of the photomultiplier from the sample. (c) CD values one or two orders of magnitude larger than normal. Figure 1 illustrates some of these anomalies (unpublished data).

Many qualitative explanations for the anomalies were proposed, including differential scattering of left and right circularly polarized light, light absorption so intense that the interior of the aggregate could no longer contribute to absorption (Duysens flattening of absorption peaks), and liquid crystal behavior (2-4, 12-15). Quantitative explanations based on phenomenological equations were also presented. The optical

properties of the sample were related to macroscopic parameters, such as the refractive index and absorption coefficient of its constituents, but the measured CD could not be related to molecular properties. (13, 16-19).

Recently, a new quantitative understanding of differential scattering of circularly polarized light has been obtained. We were able to relate the difference in scattering efficiency for incident left and right circularly polarized light to the detailed structure of the scattering particle (20-24). We have measured the angular dependence of this circular intensity differential scattering for a helix of known structure, and obtained good agreement with theory (25). Thus, we can now explain the "anomalous" behavior of chiral macromolecules and aggregates. We find that the scattering "artifacts" can provide valuable information about the configuration (left- or right-handed) of the component particles.

Here we will show how the differential scattering of left and right circularly polarized light contributes to the differential extinction of circularly polarized light as routinely measured in a circular dichroism spectrometer. As the differential absorption of circularly polarized light can be measured by fluorescence detected circular dichroism in a separate experiment (9,20), therefore the circular differential scattering can be obtained by difference. This circular differential scattering is directly

related to the geometry (distances and orientations between the scattering elements); it can thus give information about the conformations of the macromolecules in the sample.

THEORY

Phenomenological Equations. We wish to derive the combined effect of circular differential absorption and circular differential scattering on circularly polarized light incident on a sample. The results will be obtained in terms of $(a_L - a_R)$, the circular differential absorption, $(s_L - s_R)$, the circular differential scattering, and σ_L, σ_R, the angular-dependent scattering cross-sections for circularly polarized light.

The scattering of a sample depends on the angle between the incident beam and the scattered beam; it can be characterized by a scattering cross-section per scattering solute molecule. The solvent scattering is usually subtracted from the sample scattering, therefore the scattering cross-section characterizes the scattering of the solute molecule compared to an equal volume of solvent. In dilute solutions the scattering cross-section per molecule is independent of concentration; i.e., multiple scattering effects can be neglected. For an oriented sample the scattering cross-section will depend on the direction of incidence of the incident light, but we will consider here only unoriented samples. For a chiral, unoriented sample the scattering cross-

section will depend on the state of circular polarization of the light.

The Beer-Lambert law defines an extinction coefficient, ε.

$$I = I_o 10^{-\varepsilon c \ell}$$

Here I is the transmitted intensity when light of intensity I_o is incident on a sample with concentration, c, in pathlength, ℓ. The units of ε are $\ell\ \text{mole}^{-1}\text{cm}^{-1}$. The extinction coefficient is the sum of an absorption coefficient, a, and a scattering coefficient, s; each has units of $\ell\ \text{mole}^{-1}\text{cm}^{-1}$. These coefficients are related to molecular cross-sections for absorption, C_{abs}, and scattering, C_{sca}, by

$$a = \frac{N_o C_{abs}}{2303} \qquad\qquad s = \frac{N_o C_{sca}}{2303}$$

where N_o is Avogadro's number, and C_{abs} and C_{sca} have units of cm^2 molecule^{-1}. The molecular scattering cross-section is the integral over all angles of the angular-dependent cross-section $\sigma(\theta, \phi)$ for the scattering particle.

$$C_{sca} = \int_0^{2\pi} \int_0^{\pi} \sigma_{sca}(\theta, \phi) \sin\theta d\theta d\phi \qquad (1)$$

The angular-dependent scattering cross-section has units of cm^2

molecule^{-1} and characterizes the intensity scattered at any angle by a molecule.

$$I_{sca}(\theta,\phi,r) = \frac{\sigma_{sca}(\theta,\phi)I}{r^2} \qquad (2)$$

The scattered intensity depends on the light incident on the molecule, I, the distance, r, from the scattering molecule, and the angles θ,ϕ measured relative to the incident beam. For an unoriented sample, in which the molecules have all orientations, the molecular scattering cross-section depends on only one angle, $\sigma_{sca}(\theta)$. The measured scattered intensity will depend on the experimental arrangement through r, the distance from the sample to detector, the angular acceptance of the detection optics, and the scattering volume as defined by the incident beam and the detection optics. The measured intensity will of course depend on the concentration of the sample. We will designate the angular-dependent scattering cross-section from the scattering volume (which depends on concentration and detection geometry) as $\sigma(\theta)$; it has units of cm^2.

$$I_{sca}(\theta) = \frac{\sigma(\theta)I}{r^2} \qquad (3)$$

The Transmitted Beam. Consider a small disk-shaped scattering volume in the center of a cylindrical cell (Figure 2). A detector placed in front of the cell to measure the transmitted beam will also sense light scattered at zero degrees. Applying the Beer-Lambert law, we obtain

$$I = I_o 10^{-\varepsilon c \ell} + \frac{\sigma(0)}{r^2} I_o 10^{-\varepsilon c} \qquad (4)$$

In the second term we have taken into account the attenuation of the incident beam before it reaches the scattering volume, and the attenuation of the scattered beam before it leaves the cell; $\sigma(0)$ is the scattering cross-section in the forward direction and r is the distance from the scattering volume to the detector. Standard circular dichroism spectrophotometers measure a signal proportional to $(I_L - I_R)/(I_L + I_R)$ where I_L and I_R are the intensities detected when left and right circularly polarized light is incident. As derived in the Appendix for the usual approximation that the circular dichroism is small, the signal measured along the transmitted beam is

$$\frac{I_L - I_R}{I_L + I_R} = \frac{-2.303(\varepsilon_L - \varepsilon_R)c\ell}{2} + \frac{\sigma_L(0) - \sigma_R(0)}{2r^2 + \sigma_L(0) + \sigma_R(0)} \qquad (5)$$

The first term is the usual one considered; however, we must keep in mind that this term has two contributions: absorption and scattering.

$$\varepsilon_L - \varepsilon_R = (a_L - a_R) + (s_L - s_R) \qquad (6)$$

The circular differential scattering coefficient $(s_L - s_R)$ is related to the integral over all angles of the angular-dependent scattering cross-sections.

$$s_L - s_R = \frac{N_o}{2303} \int_0^{2\pi} d\phi \int_0^{\pi} [\sigma_{sca,L}(\theta) - \sigma_{sca,R}(\theta)] \sin\theta d\theta \qquad (7)$$

The second term in Eq. (5) represents the contribution from the light scattered forward along the incident beam. This contribution depends on the size and position of the photomultiplier detector. We will usually try to make this term negligible by having the detector far from the cell (r large), and by having a small acceptance angle for the detector [$\sigma(0)$small]. If this term is not small, it can have either sign relative to $(s_L - s_R)$. The sign of the circular intensity differential scattering at zero degrees is not simply related to the sign of the circular differential scattering coefficient $(s_L - s_R)$, which depends on differential scattering at all angles.

The Scattered Beam. At any angle except zero, only the scattered beam is detected. The derivation of the circular intensity differential scattering is straight forward (see Appendix), however one must realize that the scattered beam is not

circularly polarized except at 0° and 180°. Although we do not measure the polarization of the scattered beam, its attenuation in passing through the cell does depend on its state of polarization. When a left circularly polarized beam is incident on a point scatterer. the scattered light will be left circularly polarized at 0°, linearly polarized at right angles (90°) and right circularly polarized at 180°. At any angle in between. the light is elliptically polarized, but its state of polarization depends only on the scattering angle. For larger scatterers the state of polarization also depends on the properties of the scattering molecule; however. for most samples the depolarization effect is much less than 5%. We will assume that it is negligible. and obtain (see Appendix)

$$\frac{I_L - I_R}{I_L + I_R} = \frac{-2.303}{2}(\varepsilon_L - \varepsilon_R)c\ell\left[\frac{(1 + \cos\theta)^2}{2(1 + \cos^2\theta)}\right] + \frac{\sigma_L(\theta) - \sigma_R(\theta)}{\sigma_L(\theta) + \sigma_R(\theta)} \quad (8)$$

This is an expression for the circular intensity differential scattering at any angle. Again there are two terms. The first term characterizes the differential attenuation of the scattered beam due to circular differential absorption and scattering in the cell. The second term characterizes the circular differential scattering cross-sections at any angle. The terms can be separated by varying the concentration; the first term is linear in the concentration. so extrapolation to zero concentration

leaves only the second term, which is independent of concentration. In our earlier work (21-24) we have only considered this second term. Outside all absorption bands ($a_L - a_R$) is zero and only ($s_L - s_R$) contributes to the first term. However, as mentioned before, the sign and magnitude of ($s_L - s_R$) is not simply related to $\sigma_L(\theta) - \sigma_R(\theta)$.

Although we are primarily interested here on the effects of circular differential scattering, Eq. (8) can be applied to a non-chiral scatterer mixed with a chiral absorber (for example, polystyrene spheres in a camphor sulfonic acid solution). Then only the first term in Eq. 8 is non-zero. This method can be called <u>scattering detected circular dichroism</u> in analogy with fluorescence detected circular dichroism (26).

Molecular Parameters. Equation (7) relates the circular differential scattering coefficient to the integral over the angular-dependent scattering cross-sections. These cross-sections have been previously derived from molecular properties -- the relative orientations and distances between polarizable groups in the molecule [see Eq. (12) of ref. 24].

$$\sigma_L(\theta) - \sigma_R(\theta) = -\frac{\pi^2}{2\lambda^4} \sum_i \sum_j \mathrm{Re}(\alpha_i \alpha_j^*) \underset{\sim}{e}_i \times \underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij} \{(\underset{\sim}{e}_i \cdot \underset{\sim}{e}_j)(\frac{j_2}{q} - j_1)$$

$$- (\underset{\sim}{e}_i \cdot \hat{\underset{\sim}{R}}_{ij})(\underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij})(\frac{5j_2}{q} - j_1)\} (\sin^3\frac{\theta}{2} + \sin\frac{\theta}{2}) \quad (9)$$

where $j_1(q) \equiv \sin q/q^2 - \cos q/q$ and $j_2(q) \equiv (3/q^3 - 1/q)\sin q - 3 \cos q/q^2$, are the first and second order spherical Bessel functions respectively. $\theta/2$ is one-half of the scattering angle and the argument of the spherical Bessel function is:

$$q \equiv \frac{4\pi R_{ij}}{\lambda} \sin\frac{\theta}{2}$$

where R_{ij} is the distance between groups i and j in the molecule and λ is the wavelength of the incident light in the medium. The unit vectors $\underset{\sim}{e}_i$ and $\underset{\sim}{e}_j$ specify the directions of the principal axes of the polarizabilities α_i and α_j; $\hat{\underset{\sim}{R}}_{ij}$ is the unit vector from group i to j. Re means that the real part of the expression in parentheses is used; this expression governs the wavelength dependence of the scattering. The integration of Eq. (9) as indicated by Eq. (7) can be performed analytically; the results are:

$$s_L - s_R = \frac{-4\pi^3 N_o}{2303\lambda^4} \sum_i \sum_j \mathrm{Re}\ (\alpha_i \alpha_j^*)(\underset{\sim}{e}_i \times \underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij})\{(\underset{\sim}{e}_i \cdot \underset{\sim}{e}_j)\ f(a)$$

$$- (\underset{\sim}{e}_i \cdot \hat{\underset{\sim}{R}}_{ij})(\underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij})\ g(a)\} \qquad (10)$$

$$f(a) = \frac{16 + 8(a^2-2)\cos a + 2a(a^2-8)\sin a}{a^5}$$

$$g(a) = \frac{8(a^2+6) + 16(a^2-3)\cos a + 2a(a^2-24)\sin a}{a^5}$$

$$a = 4\pi R_{ij}/\lambda$$

The terms f(a) and g(a) are unitless functions which become zero as $\underline{a}$ approaches zero or infinity.

The sign of the differential scattering coefficient $(s_L - s_R)$ depends upon (1) the chirality of the scattering particle as characterized by the relative orientations of the individual scattering elements ($\underset{\sim}{e}_i$, $\underset{\sim}{e}_j$, $\hat{\underset{\sim}{R}}_{ij}$) and (2) the size of the particle as characterized by $\underline{a} = 4\pi R_{ij}/\lambda$. The factor which characterizes the wavelength dependence of the polarizing abilities ($\mathrm{Re}\alpha_i\alpha_j^*$) affects the magnitude of $(s_L - s_R)$, but it is always positive (see Fig. 3).

For $\underline{a}$ less than 1 (R_{ij}/λ less than 1/12.5), g(a) is negligible and f(a) can be approximated by (-a/9). Therefore, for $\underline{a}$ less than 1, a particularly simple expression can be written for $s_L - s_R$.

$$s_L - s_R = \frac{16\pi^4 N_o}{(9)(2303)\lambda^5} \sum_i \sum_j \mathrm{Re}(\alpha_i \alpha_j^*)(\underset{\sim}{e}_i \times \underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij})(\underset{\sim}{e}_i \cdot \underset{\sim}{e}_j) R_{ij}$$

(11)

For distances R_{ij} small compared to the wavelength, the differential scattering is linear in these distances, and depends on simple geometric parameters (Eg. 11). For larger distances Eq. 10 must be used. In Eq. 10 the function f(a) is negative for (R/λ) less than 1/2.3 with a minimum value of -0.17 at (R/λ) approximately equal to 1/5; it then oscillates in sign with decreasing magnitude. The function g(a) is positive for (R/λ) less than 1/1.2 with a maximum value of +0.11 at (R/λ) approximately equal to 1/2.3; it then oscillates in sign with decreasing magnitude. Therefore, the main contributions to $s_L - s_R$ will be for distances between scattering groups which vary from 1/20 to 1 times the wavelength of light. The differential scattering coefficient is thus related in a direct way to the geometry of the scatterers.

Comparison between the differential absorption coefficient and the differential scattering coefficient. Equation (10) relates the geometrical parameters of randomly oriented molecules with the observed differential scattering coefficient $s_L - s_R$. This expression is remarkably similar to that obtained in the classical theory of the differential absorption coefficient (27).

$$a_L - a_R = \frac{16\pi^3 N_o}{6909\ \lambda^2} \sum_i \sum_j \left[-\mathrm{Im}(\alpha_i \alpha_j)\right] \underset{\sim}{e}_i \times \underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij} G_{ij} R_{ij} \tag{12}$$

We have assumed that the interactions (G_{ij}) between groups are not large, so that only linear terms in the interaction are kept and the interactions are approximated by a normalized dipole interaction.

$$G_{ij} = (1/R_{ij}^3)\left[\underset{\sim}{e}_i \cdot \underset{\sim}{e}_j - 3(\underset{\sim}{e}_i \cdot \hat{\underset{\sim}{R}}_{ij})(\underset{\sim}{e}_j \cdot \hat{\underset{\sim}{R}}_{ij})\right] \tag{13}$$

This dipole interaction is an approximation to the electronic interaction between chromophores. The sign of the differential absorption coefficient depends on the chirality of the absorbing particle (characterized by $\underset{\sim}{e}_i$, $\underset{\sim}{e}_j$ and $\hat{\underset{\sim}{R}}_{ij}$). The factor which characterizes the wavelength dependence $[-\mathrm{Im}(\alpha_i \alpha_j)]$ is non-zero only in the absorption band; it is always positive on the long wavelength side of the absorption band and negative on the short wavelength side (see Fig. 3).

Comparing Eq. (12) with Eq. (11) or Eq. (10) we can see the contribution of two chromophores to differential scattering vs. differential absorption. At short distances G_{ij} is large and $s_L - s_R$ is small compared to $a_L - a_R$, but as R_{ij} increases $s_L - s_R$ becomes more important. The ratio depends on R_{ij}^3.

$$\frac{s_L - s_R}{a_L - a_R} \sim \frac{R_{ij}^3}{\lambda^3} \qquad (14)$$

This expression is valid for R_{ij} not larger than λ. It explains why only large chiral objects with dimensions larger than 1/20 the wavelength of light show measurable differential scattering effects on the circular dichroism.

The wavelength dependences of differential scattering and absorption are, of course, quite different from each other. They are characterized by the wavelength dependence of the polarizabilities. Each polarizability has an absorptive component (with the shape of an absorption band) and a dispersive component (with the shape of the wavelength dependence of the corresponding refractive index). For two identical chromophores the function, $\mathrm{Re}(\alpha_i \alpha_j^*)$, which controls the wavelength dependence of $s_L - s_R$, is the sum of the squares of the absorptive and dispersive components. The function, $[-\mathrm{Im}(\alpha_i \alpha_j)]$, which controls the wavelength dependence of $a_L - a_R$, is the product of the absorptive and dispersive components. The formalism comes from considering each polarizability to be complex, with the real part being the dispersive component and the imaginary part being the absorptive component. These functions are plotted in Fig. 3 for a typical absorption band (the long wavelength band of adenylic acid). The actual signs and magnitude of $a_L - a_R$ and $s_L - s_R$ will depend on the geometric factors that multiply these functions (see

Eqs. 10-12), but the wavelength dependence is informative. Outside the absorption bands only $s_L - s_R$ is non-zero, and its sign can be related to the chirality of the scatterer.

CONCLUSION

Circular differential scattering can be a significant contribution to circular dichroism whenever the dimension of the particles of interest are one twentieth the wavelength of light, or are larger. Whether the scattering effects are important or not will depend specifically on the shape of the particle and its polarizabilities. The sign and magnitude of the circular differential scattering depend directly on the distance and angles between the scattering units. It is not expected to be significant for unaggregated proteins or nucleic acids in solution. However, whenever an apparent circular dichroism is measured outside the absorption bands of the sample, the differential scattering must obviously be taken into account before a quantitative interpretation of the circular dichroism is attempted.

Circular differential scattering depends upon the phase difference of the light scattered from different points in the molecule, therefore to a first approximation the electronic interaction between the scattering units can be neglected. This interaction, which is required for circular differential absorption, is difficult to determine quantitatively. It is

largest for short distances between interacting units and it decreases roughly as the inverse cube of the distance. Thus circular differential absorption, which until recently was the only contribution to circular dichroism considered, reveals short-range conformations (distances less than 2-3 nm). It requires detailed knowledge about electronic transitions and electronic interactions of chromophores for quantitative calculation. Circular differential scattering, which until now was treated as an unwanted artifact, is a measure of long-range organization (distances greater than 20 nm). It can be interpreted simply and directly in terms of scattering from pairs of polarizable groups in the molecule (Eq. 10).

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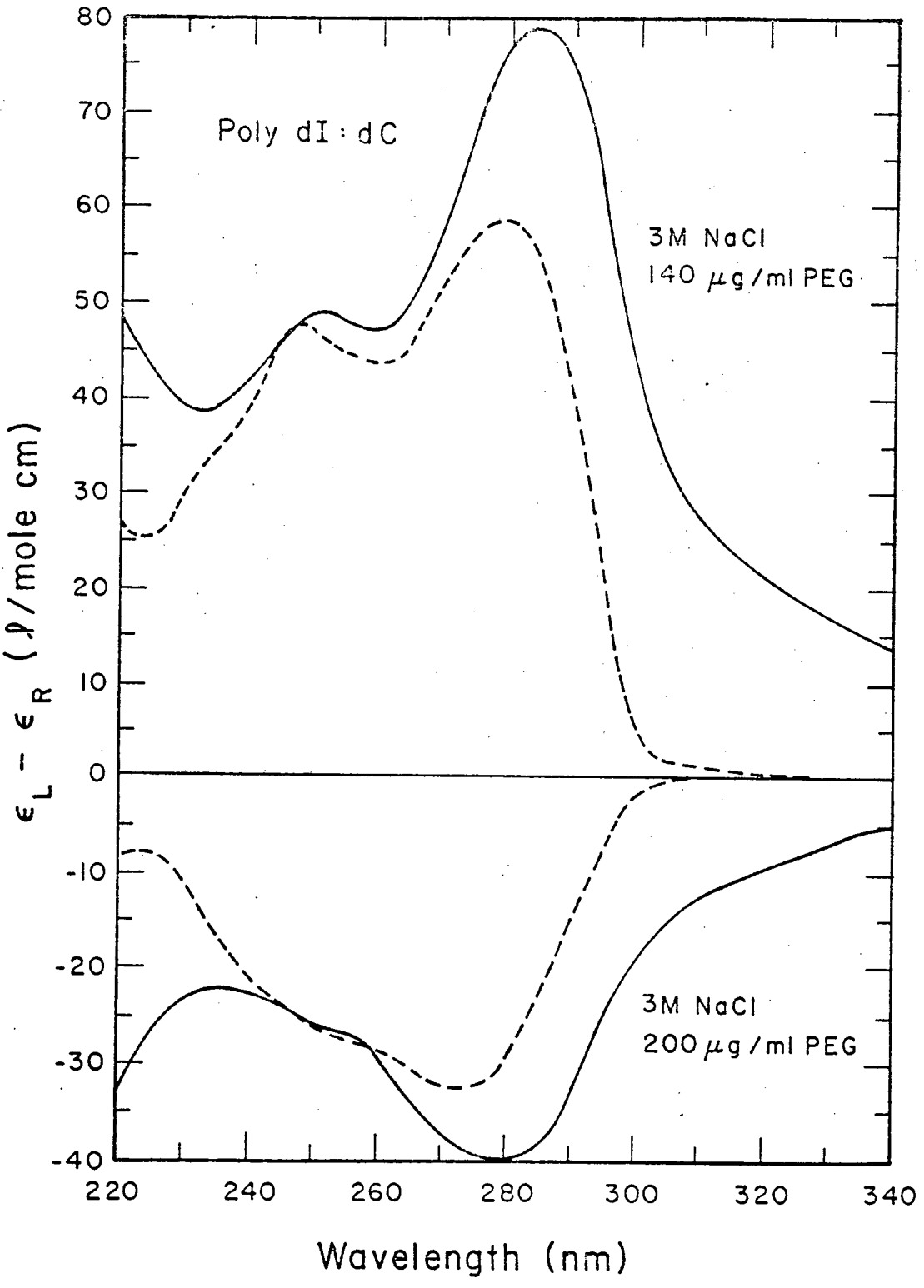
Legends for Figures:

Figure 1. An illustrative example of the effect of circular differential scattering on circular dichroism. The synthetic polynucleotide poly dI: poly dC was placed in different concentrations of polyethylene glycol in 3 M NaCl. The solid lines show the measured circular dichroism which is large at wavelengths above the absorption region of the polynucleotide, the dashed lines show the approximate removal of the differential scattering effect provided by a fluorscat cell (14). Data from Evdokimov and Maestre.

Figure 2. The scattering geometry. The scattered intensity at angle theta depends on the scattering cross-section at that angle, $\sigma(\ )$, divided by the square of the distance, r, from the scattering volume to the photomultiplier detector. The attenuation of the incident light before reaching the scattering volume, and of the scattered light before leaving the cell must be considered. As incident circularly polarized light changes its state of polarization depending on the scattering angle, the appropriate weighted extinction coefficient, $\langle\varepsilon\rangle$, must be used for the scattered beam.

Figure 3. The typical wavelength dependence for circular differential absorption, $a_L - a_R$, and circular differential scattering, $s_L - s_R$. The shape of the 260 nm absorption band of adenylic acid was used to obtain the absorptive and dispersive components of the polarizability. The calculated curves were normalized at their maximum values.



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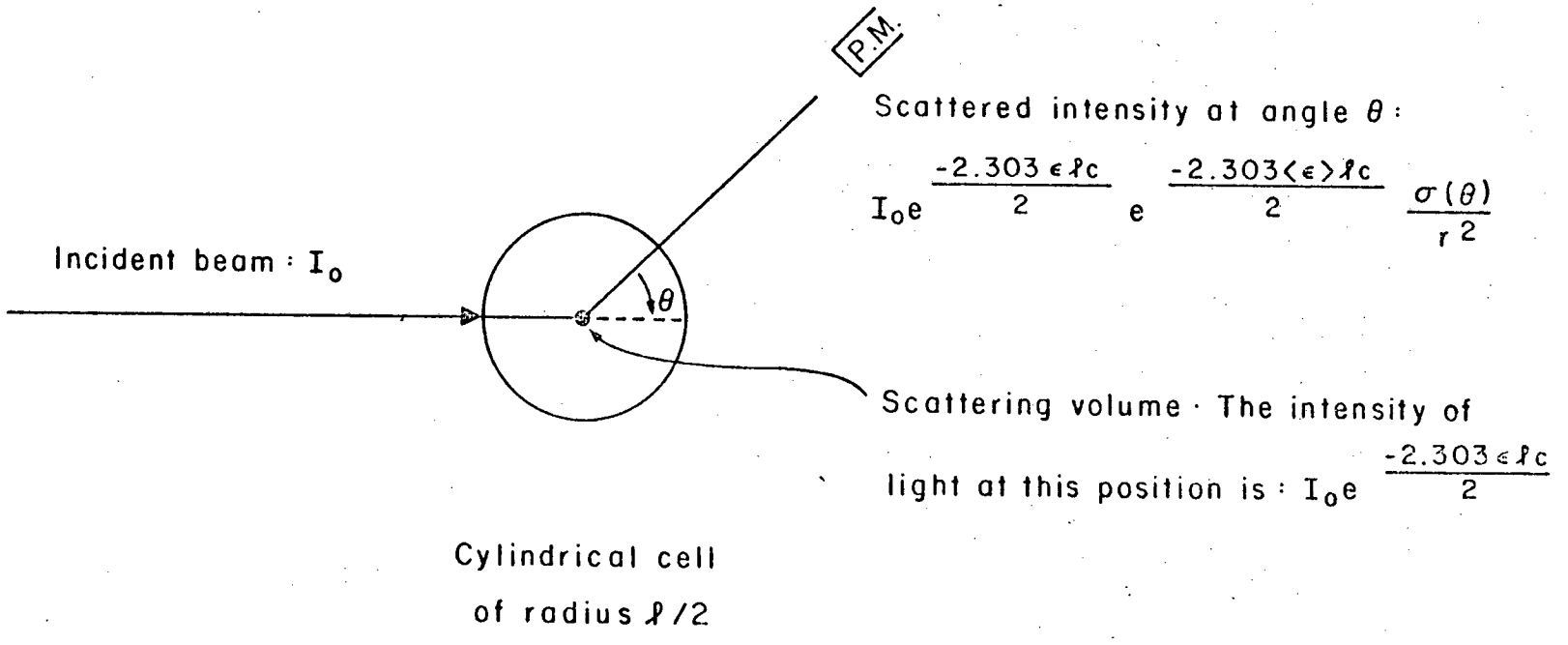

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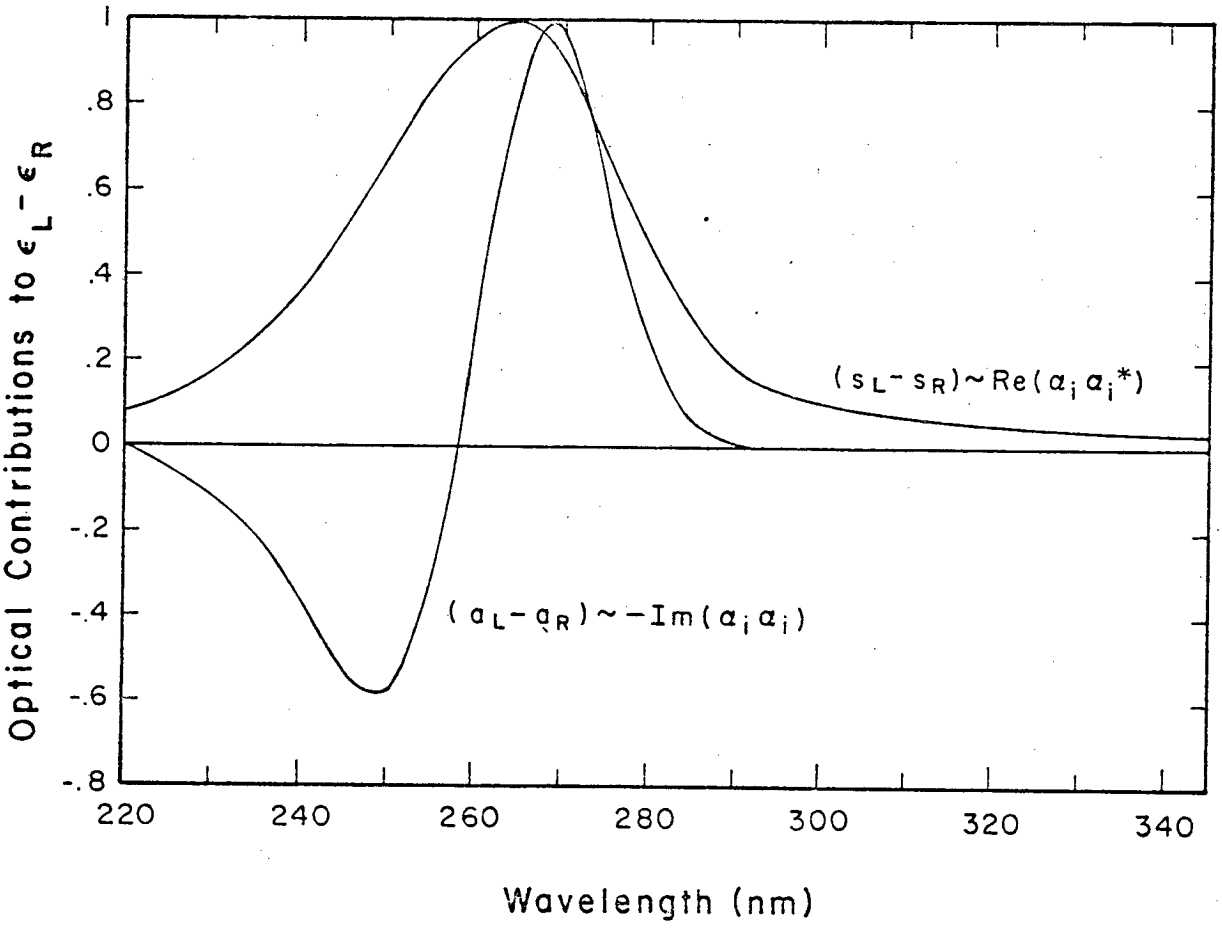

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APPENDIX

Effect of Scattering on the Transmitted Beam. A detector placed to measure the transmitted beam through a sample (the usual procedure) will also respond to scattered light along the incident beam. For left circularly polarized light incident, the detected intensity is (see Figure 2):

$$I_L = \left[1 + \frac{\sigma_L(0)}{r^2}\right] I_o e^{-2.303\varepsilon_L c} \tag{A1}$$

Using the identity $\varepsilon_L = (\varepsilon_L + \varepsilon_R)/2 + (\varepsilon_L - \varepsilon_R)/2$, we write

$$I_L = \left[1 + \frac{\sigma_L(0)}{r^2}\right] I_o e^{-2.303(\varepsilon_L + \varepsilon_R)c\ell/2} e^{-2.303(\varepsilon_L - \varepsilon_R)c\ell/2} \tag{A2}$$

Using an equivalent expression for I_R, we obtain

$$\frac{I_L - I_R}{I_L + I_R} = \frac{\left[1 + \frac{\sigma_L(0)}{r^2}\right]e^{-z} - \left[1 + \frac{\sigma_R(0)}{r^2}\right]e^{+z}}{\left[1 + \frac{\sigma_L(0)}{r^2}\right]e^{-z} + \left[1 + \frac{\sigma_R(o)}{r^2}\right]e^{+z}} \tag{A3}$$

with $z = 2.303(\varepsilon_L - \varepsilon_R)c\ell/2$. Similarly writing $\sigma_L(0)$ and $\sigma_R(0)$ as a sum and a difference, we obtain:

$$\frac{I_L - I_R}{I_L + I_R} =$$

$$\frac{\left[2 + \frac{\sigma_L(0)}{r^2} + \frac{\sigma_R(0)}{r^2}\right]\left(e^{-z} - e^{+z}\right) + \left[\frac{\sigma_L(0)}{r^2} - \frac{\sigma_R(0)}{r^2}\right]\left(e^{-z} + e^{+z}\right)}{\left[2 + \frac{\sigma_L(0)}{r^2} + \frac{\sigma_R(0)}{r^2}\right]\left(e^{-z} + e^{+z}\right) + \left[\frac{\sigma_L(0)}{r^2} - \frac{\sigma_R(0)}{r^2}\right]\left(e^{-z} - e^{+z}\right)} \qquad \text{(A4)}$$

We can rewrite this equation in terms of the hyperbolic tangent of z.

$$\frac{I_L - I_R}{I_L + I_R} = \frac{-\tanh z + \left[\frac{\sigma_L(0) - \sigma_R(0)}{2r^2 + \sigma_L(0) + \sigma_R(0)}\right]}{1 - \left[\frac{\sigma_L(0) - \sigma_R(0)}{2r^2 + \sigma_L(0) + \sigma_L(0)}\right]\tanh z} \qquad \text{(A5)}$$

In the usual approximation that the circular dichroism is small [$z = 2.303(\varepsilon_L - \varepsilon_R)c\ell/2 \leq 0.1$], we can replace tanh z by z and approximate the denominator by 1.

$$\frac{I_L - I_R}{I_L + I_R} = \frac{-2.303(\varepsilon_L - \varepsilon_R)c\ell}{2} + \frac{\sigma_L(0) - \sigma_R(0)}{2r^2 + \sigma_L(0) + \sigma_R(0)} \qquad \text{(A6)}$$

This is Eq. 5 which presents the contribution of differential scattering in the forward direction at zero degrees, $\sigma_L(0) - \sigma_R(0)$, to the signal as usually measured.

Effect of Scattering and Absorption on the Scattered Beam. The amount of attenuation of a scattered beam by the solutions depends on the state of

polarization of the scattered light. The scattered light will in general be elliptically polarized and we can characterize it as a sum of left and right circularly polarized components. The extinction coefficient which determines the attenuation of the scattered beam as it passes through the solution is a weighted average of ε_L and ε_R. For left circularly polarized light incident on the cell, the amptitude of the left circularly polarized component is $(1 + \cos\ \theta)/2$ and that of the right circularly polarized component is $(\cos\ \theta - 1)/2$. The appropriate extinction coefficient which depends on the weighted intensities of the components is

$$\langle\varepsilon\rangle = p\varepsilon_L + (1 - p)\varepsilon_R$$

$$p = \frac{(1 + \cos\ \theta)^2}{2(1 + \cos^2\ \theta)}$$

We thus write the intensity of the scattered beam when left circularly polarized light is incident as

$$I_L = \frac{\sigma_L(\theta)}{r^2} I_o\, e^{-2.303\varepsilon_L c\ell/2}\, e^{-2.303[p\varepsilon_L + (1 - p)\varepsilon_R]c\ell/2} \qquad \text{(A7)}$$

Proceeding as before

$$I_L = \frac{\sigma_L(\theta)}{r^2} I_o\, e^{-2.303(\varepsilon_L + \varepsilon_R)c\ell/2}\, e^{-2.303p(\varepsilon_L - \varepsilon_R)c\ell/2} \qquad \text{(A8)}$$

This is of the same form as Eq. (A2), so the same derivation yields Eq. 8 of the text.

$$\frac{I_L - I_R}{I_L + I_R} = \frac{-2.303(\varepsilon_L - \varepsilon_R)c\ell}{2}\left[\frac{(1 + \cos\theta)^2}{2(1 + \cos^2\theta)}\right] + \frac{\sigma_L(\theta) - \sigma_R(\theta)}{\sigma_L(\theta) + \sigma_R(\theta)} \qquad (A9)$$

This equation relates the circular differential scattering at any angle (except zero degrees) to differential scattering coefficients, $\sigma_L(\theta)$, $\sigma_R(\theta)$, and the circular dichroism, $(\varepsilon_L - \varepsilon_R)$. The two contributions can be separated by using the concentration dependence. The first term is linear in concentration; the second term is independent of concentration. For backward scattering ($\theta \to 180°$) the first term approaches zero. As left circularly polarized light becomes right circularly polarized when it is scattered backwards (and vice versa), there is no effect of circular dichroism. The second term in Eq. (A9) differs from the corresponding term in Eq. (A6) by the lack of $2r^2$ in the denominator. In Eq. (A6) the second term gives the contribution of zero-angle scattering to the transmitted beam; this contribution depends on the distance r. In Eq. (A9) the second term represents only the scattered beam; its effect is independent of distance.

Our expression for p, the angular dependence of the first term in Eq. (A9), only represents the effect of the transversality of light. We assume the scatterer is a point. For a realistic scatterer the state of polarization of the scattered light and the angular dependence of p will depend on the scatterer. However, the change will usually be negligible.

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